REMARKS

Claim 1 has been amended by specifying that the measurement in step (a) takes place in the presence of EDTA. Support for this amendment can be found in throughout the specification, e.g., Table 2 on page 13 and page 14, lines 15-26. Claim 1 has also been amended to measurement is performed by means of at least one monoclonal or polyclonal antibody or fragment thereof in which the antibody or fragment thereof has the specified properties. Support for this amendment can be found in original claims 3 and 5-7. Claim 1 has further been amended to specify measurement and determination in the optional clause to provide antecedent basis for language that appears later in the claim. Finally, claim 1 has been amended to clarify the language of the claims.

Claim 9 has been amended so that the kit includes EDTA. Support for this amendment can be found in throughout the specification, e.g., Table 2 on page 13 and page 14, lines 15-26. In addition, claim 9 has also been amended to indicate that the capture reagent and detecting reagent are a "first" capture reagent and a "first" detecting reagent in view of claim 10 which includes another capture reagent and detecting agent. Claim 9 has also been amended to specify the properties of the antibody or antibody fragment. Support for this amendment can be found in original claims 3, 5-7 and 12-14. Claim 9 had further been amended to use terms for which antecedent basis is present. Finally, claim 9 has been amended to clarify the language of the claims.

Claim 10 has been amended to be consistent with amended claim 9, to specify that the "another" capture reagent and the detecting agent are a "second" capture reagent and a "second" detecting reagent, respectively, and to provide proper antecedent basis.

Claim 11 has been amended to be consistent with amended claim 9.

In view of the amendments to claims 1 and 9, claims 3, 5-7, 12-14, 16, and 18-20 have been canceled. In addition, claims 4, 8 and 17 have been amended to depend from non-canceled claims.

New claims 21-27 have been added to depend on various of the amended or new claims and find support in the original claims.

It is submitted that these amendments do not constitute new matter and their entry is requested.

The Examiner rejected claims 1-10 under 35 U.S.C. § 112, first paragraph for lack of enablement. The Examiner contends that the specification is enabling for the assessment of bone fragility and bone fracture risk but is not enabling for assessment of osteoporosis. It is submitted that the Examiner is in error in this rejection.

The present invention is directed to measuring γ-carboxylated osteocalcin, i.e. osteocalcin carboxylated with one to three carboxyls, in the presence of EDTA to eliminate the detrimental effect of bivalent metal ions, such as Ca^{2+}, on the specificity of the affinity of the monoclonal or polyclonal antibody or fragment towards γ-carboxylated osteocalcin. The γ-carboxylated osteocalcin is measured using at least one monoclonal or polyclonal antibody or fragment thereof being specific for gamma-carboxylated osteocalcin recognizing an epitope occurring in the region of the amino acids 17-24 of the gamma-carboxylated osteocalcin molecule, or being an antibody or fragment recognizing the tertiary structure associated with the gamma-carboxylated osteocalcin; and being an antibody or fragment whose specificity for gamma-carboxylated osteocalcin is dependent on the presence of bivalent metal ions, said specificity decreasing in the presence of said metal ions.

The specification clearly shows that EDTA is used to improve the specificity of the antibodies towards γ-carboxylated osteocalcin. The specification further shows that this improvement enables the use of the assay for the assessment of bone fragility and fracture risk, or osteoporosis in a person.

For example, the two-site assays #2, #4 and #9 disclosed in the application have been shown, through there characterization on page 12, lines 16 to 28, to be highly applicable for determination of (a) intact human osteocalcin (IOC) irrespective of the degree of carboxylation (assay #2), (b) total human osteocalcin (TOC) irrespective of the degree of carboxylation (assay #4), and γ-carboxylated osteocalcin (COC) (assay #9).

The results shown in Table 2 on page 13 of the application, confirm that assay #9, performed in the presence of EDTA, employs a monoclonal antibody highly specific for COC as characterized above.

The results shown in Table 3 on page 16 of the application, show that assay #9, using EDTA (as noted on page 14, lines 15-26, particularly line 19) could very efficiently differentiate between the effect of warfarin on γ-carboxylated osteocalcin levels of warfarin (which is known to affect these levels) treated patients compared to controls. These results demonstrate that the assay of the method according to the invention is highly specific even in practice.

The results of a study in elderly people as a whole disclosed on pages 18 to 24 of the application show that the values of COC and especially the COC/TOC ratio and COC/IOC ratio do predict the risk of fracture related to bone fragility and osteoporosis in these people. A comparison of the results shown in Table 6 on page 21 of the present application with the results shown in Table 1 on page 7 of Delmas et al. (EP 0 557 663) clearly shows that the present invention gives a more reliable prediction of fracture risk in both genders. This more reliable prediction results from conducting the assay in the presence of EDTA which increases the specificity of the antibodies to γ-carboxylated osteocalcin.

Applicants note that it is generally accepted in the art that bone fragility and fracture highly correlates with osteoporosis. See, e.g., Delmas et al. (EP 0 557 663; US 6,004,765). This correlation is accepted in the art irregardless of any possible controversy in the art concerning an association of osteoporosis and γ-carboxylated osteocalcin. Thus, the assessment of bone fragility and bone fracture is also associated with the assessment of osteoporosis. The present application clearly demonstrates the assessment of bone fragility and fracture risk. Therefore, in view of the accepted correlation in the art, the present application also clearly demonstrates the assessment of osteoporosis.

Applicants suspect that the controversy concerning a correlation between osteoporosis and γ-carboxylated osteocalcin may very well be the result of assay methods in the prior art that were not sufficient for assessing the concentration of γ-carboxylated osteocalcin. The present invention provides an assay that is highly sensitive to γ-carboxylated osteocalcin and has demonstrated the correlation between γ-carboxylated osteocalcin and bone fragility and fracture risk, and hence osteoporosis. The specification provides clear guidance for evaluating osteoporosis using the

improved assay. That is, the specification specifically states that a lower COC in a person than the mean COC for the general population is an indication of osteoporosis. The specification also specifically states that a lower COC/IOC ratio or COC/TOC ration in a person than the mean COC/IOC ratio or COC/TOC ration for the general population is an indication of osteoporosis. With these specific statements in the specification and the specific teaching of the improved assay, Applicants submit that clear guidance and instructions have been provided to a skilled artisan to practice the claimed invention. Thus, Applicants submit that no undue experimentation would be required to practice the claimed invention.

In view of the above remarks, it is submitted that the claims are fully enabled by the specification. Withdrawal of this rejection is requested.

The Examiner rejected claims 1-20 under 35 U.S.C. § 112, second paragraph for being indefinite. Claims 1 and 9 have been amended to clarify the language of the claim.

In view of the amendments to the claims and the above remarks, it is submitted that the claims are definite. Withdrawal of this rejection is requested.

The Examiner rejected claims 9-20 under 35 U.S.C. § 103 (a) as being obvious over Koyama et al. (J Immunological Methods 139:17-23, 1991). It is submitted that the amended claims are not obvious from the teachings of Koyama et al.

The present invention is directed to measuring γ-carboxylated osteocalcin, i.e. osteocalcin carboxylated with one to three carboxyls, in the presence of EDTA to eliminate the detrimental effect of bivalent metal ions, such as Ca^{2+}, on the specificity of the affinity of the monoclonal or polyclonal antibody or fragment towards γ-carboxylated osteocalcin. The γ-carboxylated osteocalcin is measured using at least one monoclonal or polyclonal antibody or fragment thereof being specific for gamma-carboxylated osteocalcin recognizing an epitope occurring in the region of the amino acids 17-24 of the gamma-carboxylated osteocalcin molecule, or being an antibody or fragment recognizing the tertiary structure associated with the gamma-carboxylated osteocalcin; and being an antibody or fragment whose specificity for gamma-carboxylated osteocalcin is dependent on the presence of bivalent metal ions, said specificity decreasing in the presence of said metal ions. The

specification clearly shows that EDTA is used to improve the specificity of the antibodies towards γ-carboxylated osteocalcin. The specification further shows that this improvement enables the use of the assay for the assessment of bone fragility and fracture risk, or osteoporosis in a person.

Koyama et al. discloses the use of monoclonal antibodies specific for fully carboxylated osteocalcin in an osteocalcin assay as noted in Delmas et al. (EP 0 557 663; page 2, lines 40 and 41). Koyama et al. does not disclose or suggest the use of EDTA to improve the specificity of the antibodies towards carboxylated osteocalcin and does not disclose or suggest the use of the assay for the assessment of bone fragility and fracture risk, or osteoporosis in a person. Thus, it is submitted that Koyama et al. does not render the claimed subject matter obvious.

In view of the amendments to the claims and the above remarks, it is submitted that the claimed subject matter is not obvious over Koyama et al. Withdrawal of this rejection is requested.

In view of the above amendments and remarks, it is believed that the claims satisfy the requirements of the patent statutes and are patentable over the prior art. Reconsideration of the instant application and early notice of allowance are requested. The Examiner is invited to telephone the undersigned if it is deemed to expedite allowance of the application.

Respectfully submitted,

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